Multichannel thin-film electrode for intramuscular electromyographic recordings

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Farina D, Yoshida K, Stieglitz T, Koch KP. Multichannel thin-film electrode for intramuscular electromyographic recordings. J Appl Physiol 104: 821–827, 2008. First published November 29, 2007; doi:10.1152/japplphysiol.00788.2007.—It is currently not possible to record electromyographic (EMG) signals from many locations concurrently inside the muscle in a single wire electrode system. We developed a thin-film wire electrode system for multichannel intramuscular EMG recordings. The system was fabricated using a micro-machining process, with a silicon wafer as production platform for polyimide-based electrodes. In the current prototype, the flexible polymer structure is 220 µm wide, 10 µm thick, and 1.5 cm long, and it has eight circular platinum-platinum chloride recording sites of 40-µm diameter distributed along the front and back surfaces with 1,500-µm intersite spacing. The system prototype was tested in six experiments where the electrode was implanted into the medial head of the gastrocnemius muscle of rabbits, perpendicular to the pennation angle of the muscle fibers. Asynchronous motor unit activity was induced by eliciting the withdrawal reflex or sequential crushes of the sciatic nerve using a pair of forceps. Sixty-seven motor units were identified from these recordings. In the bandwidth 200 Hz to 5 kHz, the peak-to-peak amplitude of the action potentials of the detected motor units was 75 ± 12 µV and the root mean square of the noise was 1.6 ± 0.4 µV. The noise level and amplitude of the action potentials were similar for measures separated by up to 40 min. The experimental tests demonstrated that thin film is a promising technology for a new type of flexible-wire intramuscular EMG recording system with multiple detection sites.

since the first recordings of motor unit action potentials in humans (2), it has become possible to decode the neural input to the muscle through the analysis of individual motor unit electrical activities from intramuscular electromyographic (EMG) recordings, with either indwelling wire or needle electrodes. The detection of electric signals from muscles provides a window into the neural output from the spinal cord because the discharges of the motoneuron are directly linked to the action potentials of the innervated muscle fibers. Thus, the muscle fiber electrical activity can be seen as the output layer of the spinal neural circuitry.

In vivo identification of extracellular action potentials in multiunit EMG recordings has allowed the assessment of the discharge pattern of motoneurons from a specific area in the muscle near the recording site of a highly selective electrode. The selectivity of the recording is necessary for the identification of individual motor units from the multiunit recordings; however, there is a limit to the number of motor units that can be investigated concurrently. For this reason, most in vivo studies report results on few motor units that constitute only a small proportion of the population of active motor units from a relatively small muscle area (6). Most of the knowledge on motor unit physiology is based on the interpretation of ensembles of serially recorded single-unit activity from different sessions and subjects. Such recordings enable the development of a generalized scheme of muscle control, although some limitations remain.

The detection of signals from many locations in the muscle, which is known as spatial sampling, is a viable strategy for increasing the number of detected sources and the sampled muscle area. The loss of information determined by high selectivity in each detection location is compensated for by sampling many muscle regions. This approach has been applied in surface EMG recordings (15, 17). Multichannel needle electrodes and multiwire electrodes have also been applied (1, 3) but with detection surfaces closely spaced between each other, thus recording the same motor unit activities from slightly different locations. Moreover, the needle electrode systems are impractical in many applications, such as dynamic contractions, and are uncomfortable for the subject.

Similar issues have been faced in nerve recordings where multiple sites allow the sampling of subpopulations of fibers conveying information to and from the limb. Longitudinal intrafascicular electrodes (LIFEs) are fine-wire electrodes designed to be implanted into peripheral nerves (10, 14). Conventional LIFEs are constructed using Teflon-insulated platinum-iridium wires (9) or metallized and insulated polypyrind filaments (12). The number of wires that can be attached to the tungsten needle limits the number of recording channels. The limit is two channels for Pt-Ir electrodes (22). To expand this capability, our group has experimented with microfabricated thin-film LIFEs for nerve recordings (24). The thin-film technology allows the design of multiple detection sites with consistent site geometry on a flexible substrate that is relatively small. This technology has not yet been applied to intramuscular electrodes. This paper describes the development and test of a prototype of thin-film system for intramuscular motor unit
recordings. The system is currently limited to eight channels, as a proof of principle, and was tested on animals.

MATERIALS AND METHODS

Intramuscular thin-film electrode. The thin-film electrode system was fabricated using microfabrication techniques. A silicon wafer was used as production platform for the polyimide-based electrodes (19). As a base, a 5-μm-thick layer of polyimide was spin coated on the wafer. A photoresist was then deposited and structured by photolithography. Connection pads, electrodes, and conductive tracks, which connected the electrodes to the connector, were deposited by platinum sputtering. The spare metal was removed by a lift-off process. A second polyimide layer insulated the tracks. To open the electrodes and the contacts, an aluminum mask was used for selective reactive ion etching.

The structure of the system is shown in Fig. 1. The device is 220 μm wide, 10 μm thick, and it has eight circular platinum-platinum chloride recording sites, each with a diameter of 40 μm distributed along the front and back surfaces, with 1,500-μm intersite spacing. All tracks are 10-μm wide, 300-nm thick, and made of platinum. This leads to an average track resistance of ~600 Ω, which is small compared with the interface impedance of the small electrode contacts.

The adapter from the electrode to the amplifier is a laser cut ceramic substrate (700 μm thick, 19.8 × 4.3 mm size) with screen printed silver-palladium tracks and gold pads. The pads of the thin-film electrode are bonded onto the ceramic substrate by Microflex bonding technique (13). The tracks consist of silver-palladium. Both ends of the flexible polyimide-based system are bonded onto one common ceramic adapter after folding the wire system at the center line (Figs. 1 and 2).

Animal preparation. The thin-film system was tested in six experiments on rabbits. The aim of the tests was to determine whether it was possible to record motor unit action potentials with the developed thin-film electrode. Experiments were conducted on anesthetized adult female New Zealand White rabbits (~4.3 kg) under protocols approved by the Danish Committee for the Ethical Use of Animals in Research. The rabbits were anesthetized using intramuscular injections of 0.15 mg/kg midazolam (Dormicum, Alpharma, Oslo, Norway), 0.03 mg/kg fentanyl, and 1 mg/kg flurane (combined in Hypnorm, Janssen Pharmaceutica, Beerse, Belgium), and they were maintained with regular injections of the same anesthesia every 20 min. The depth of anesthesia was assessed by monitoring the heart rate and blood oxygen saturation. A Steinmann bone pin was placed near the distal epiphysis of the left femur and was used to anchor the preparation to the experimental apparatus. The thin-film electrode system was implanted into the medial head of the gastrocnemius muscle, perpendicular to the pennation angle of the muscle fibers.

Insertion method. The microfabricated thin-film electrode was connected to an 80-μm-diameter, 1.5-cm-long tungsten needle by several strands of polyaramid filaments that were glued to the needle using a cyanoacrylate adhesive, as shown in Figs. 2 and 3 (7, 23). A surgical incision was made in the skin directly overlying the insertion site, and the skin was retracted to expose the belly of the muscle. Under the stereomicroscope, the fiber orientation of the intended implant site was visualized. The tungsten needle, to which the thin-film structure was attached by the polyaramid filaments, was inserted through the epimysium and parmyosum, and it was threaded through the endomysium, approximately perpendicular to the fiber orientation, for ~8 mm before it was directed out of the muscle. Figure 2 shows schematically the entry and exit points of the needle and the position of the thin-film system inside the muscle. The needle was then pulled to draw the thin-film electrode into the muscle via the polyaramid filament that connected the electrode to the needle. The needle was pulled in a way that the eight recording sites of the thin film were centered between the entry and exit points into and out of the muscle (Fig. 2). The needle and the polyaramid filament thus served only to introduce the thin film into the muscle. The polyaramid filament was cut after the insertion, the needle was removed, and the thin-film structure was left in the muscle for signal recording. The electrode was stabilized by sewing the connector to the animal skin (Fig. 2).

Experimental procedure. Asynchronous motor unit activity was induced by sequentially eliciting the withdrawal reflex or by crushing the sciatic nerve using a pair of forceps. The withdrawal reflex was induced by painful stimulation at the paw, either by pinching the skin webbing between the digits or by pricking the plantar surface of the foot with a needle. In some cases it was not possible to induce a withdrawal reflex and only the responses to the nerve crushes were recorded. Because crushing damages the nerve fibers, subsequent crushes were made from proximal to distal locations along the nerve. The level of anesthesia before the first nerve crush was increased to the point where the animal did not react to the nerve crush.

The EMG signals were amplified and filtered using ultra-low-noise preamplifiers (AI402, Axon Instruments) attached to an eight-channel main amplifier (Cyberamp 380, Axon Instruments). The differential preamplifiers were connected to the electrodes in monopolar recording configuration with respect to a common, distant indifferent electrode. The gain and filter characteristics of this configuration were as follows: gain 5,000, high-pass filter 1 Hz (2nd-order Bessel), low-pass filter 20 kHz (1st-order Bessel).
A customized eight-channel digital tape recorder (ADAT-XT, Alesis) was used to acquire and digitally store the signals for offline analysis. All channels on the digital tape recorder were sampled simultaneously at 48 kHz. Although eight channels were available on the electrode system, only four channels were recorded at any time because of the limited number of ultra-low-noise preamplifiers. However, all detection sites of the system were tested in each experimental session by recording the responses from different groups of four channels.

In the first four experiments, four to five contractions were elicited with withdrawal reflexes or by crushing the sciatic nerve. In the last two experiments (long term), the recordings were performed three times with a separation of 20 min between each set of recordings, for a total of 40-min separation between the first and last set of measures during which the electrode system remained in place. In these long-term experiments, two contractions were elicited for each set of recordings. During the 20 min that separated consecutive sets of measurements, the leg of the rabbit was passively extended and flexed (manually by the operator) in the entire range of motion at a speed of 1 cycle/s for 20 times. After this maneuver and before recording, the leg was repositioned at approximately the initial position. The aim of these recordings was to assess the stability of the system over time and following slow movements of the leg.

After production, the integrity of the thin-film system was tested under different sterilization protocols that employed the use of an ultrasonic cleaner, Liquinox (Alconox), ethanol (reagent grade, Fisher Scientific), and ultrapure water (Milli-Q, Millipore). All experimental measures described above were performed after the system was tested for resistance to these sterilization procedures. In addition, 2 h before the last two experiments the thin-film system was sterilized in an autoclave, because it is usually done for wire electrodes.

After completion of the experimental procedures, the animals were euthanized with an intravenously delivered overdose of pentobarbital sodium, in accordance with the approved procedure.
Signal analysis. The signals were analyzed for 4 s after eliciting each withdrawal reflex or nerve crush. The action potentials of the detected motor units were identified from the recordings with a decomposition algorithm previously described (11). This interactive algorithm includes a user interface for manually editing and verifying the results (11). The software displays a segment of the EMG signal, the templates of the action potentials of the identified motor units, the discharge patterns, and a close-up of the signal for resolving missed discharges and superimpositions. Accuracy of the automatic decomposition was achieved by inspection of the identified discharge patterns. Although the decomposition program can handle multichannel signals, in this study signal decomposition was applied to each monopolar channel independently. The decomposition results from the different channels were then merged by automatically identifying the motor units detected at more than one detection site based on the estimated discharge pattern. Discharge patterns with more than 90% discharges closer than 1 ms were considered to belong to the same motor unit detected on different channels. Motor unit action potentials identified in subsequent recordings in the same experimental session were compared by cross-correlation and merged to indicate the responses to the sequential stimuli. The procedures for merging the single-channel decomposition results were performed with custom-made algorithms developed in Matlab version 7.0 (The Mathworks, Natick, MA).

Action potentials generated by the same motor unit were averaged and characterized by the peak-to-peak amplitude in the channel with the maximum amplitude. Furthermore, the amplitude of the action potential at the neighboring recording sites was normalized (%) to the maximal peak-to-peak amplitude. The root mean square of the noise was estimated from 1-s intervals without EMG activity. The analysis of action potentials and noise was performed in two conditions: without offline filtering and after applying a digital filter with bandpass 200 Hz to 5 kHz (anticausal Butterworth filter of order 4, implemented in Matlab). In the first condition, the signals were only filtered in the bandwidth 1 Hz to 20 kHz by the analog filters in the amplifiers, whereas the second condition corresponded more closely to the filter settings usually applied for intramuscular recordings. The bandwidth 200 Hz to 5 kHz for the offline digital filter was chosen as that minimally affecting the action potential energy, as assessed by spectral analysis of the identified action potentials.

The signals recorded at the detection sites were also linearly combined to assess the effect of filtering in the spatial domain (15). Single, double, and triple derivatives were obtained. The single differential (or bipolar) detection corresponds to the difference between two signals recorded from adjacent detection sites, the double and triple differentials denote the linear summation of signals from three and four adjacent sites, respectively, with weights 1, −2, 1, and 1, −3, 3, −1. The ratio (%) between the peak-to-peak amplitude of the action potential after the derivative operation (single, double, or triple differential) and the peak-to-peak amplitude of the monopolar signal was used to quantify the effect of linearly combining signals from the detection sites. Similar quantification indexes were used to analyze signals obtained as the difference of monopolar signals recorded from the same location at the two opposite sides of the thin-film structure. Data are reported as means and SD.

RESULTS

The electrode impedance was characterized in in vitro tests in normal (0.9%) saline at 1 kHz. From a set of seven measurements, separated by ~1 min on different detection sites of one thin-film structure, the impedance was found to be 53.0 ± 17.5 kΩ. It was possible to identify repetitive activation of motor units in all experiments. The detected action potentials represented the electrical activity of a group of muscle fibers close to the detection site and belonging to the same motor unit. Figure 4 shows the identification of a motor unit from a multounit recording that followed a nerve crush. In this example, the signal was digitally filtered in the bandwidth 200 Hz to 5 kHz.

The total number of motor units decomposed from the interference monopolar EMG in the four short-term experiments and in the first set of measures of the two longer experiments was 67 (Table 1). The average number of action potentials identified for each motor unit during 4-s intervals was 48 ± 13. The maximal peak-to-peak amplitude of the identified action potentials was 80 ± 15 μV. The root mean square of the noise without offline band-pass filtering (full bandwidth 1 Hz to 20 kHz) was 5.0 ± 2.7 μV, but the noise level was reduced to 1.6 ± 0.4 μV after band-pass filtering between 200 Hz and 5 kHz (Table 1). The band-pass filtering minimally influenced the peak-to-peak amplitude of the action potentials that decreased to 75 ± 12 μV after the offline filtering.

In the two long-term experiments, two nerve crushes were performed in each of the three sets of measures. The root mean square of the noise without offline filtering was 3.3 and 2.8 μV in the two experiments for the first set of nerve crushes (Table 1). The noise level was 3.5 and 2.5 μV after 20 min (second set of nerve crushes) and 3.7 and 3.1 μV after 40 min (third set of recordings). With digital filtering in the bandwidth 200 Hz to 5 kHz, the noise root mean square was 1.6 μV and 1.2 μV (first measure), 1.5 μV and 1.5 μV (20 min after), and 1.4 μV and 1.1 μV (40 min after). Thus the noise level was approximately the same over 40 min of experiment. The number of detected motor units in the two long-term experiments was 15 in the first set of measures (Table 1), 13 (20 min after), and 17 (40 min after). The peak-to-peak amplitude of the action potentials of the detected motor units was (average over the two long-term
From the four short-term experiments and the first set of measures of the 2 longer experiments, 34 of the 67 motor units were identified by the decomposition program in all 4 channels concurrently, whereas the action potentials of the other 33 motor units were identified in only 2 or 3 channels, because of the selectivity of the recording. The amplitude of motor unit action potentials at sites adjacent to the site with maximum amplitude was 23.3 ± 43.2% of the maximum amplitude.

Figure 5 shows recordings obtained by linear combination of the signals detected from four detection sites. In the representative example of Fig. 5, the different recordings discriminated different subsets of motor units. The recordings from the first four experiments and the first set of measures of the longer experiments in which the signals from sites L1 to L4 were concurrently detected were further investigated. In these recordings, it was possible to identify a total of 30 motor units from the monopolar signal recorded at L2. The action potentials of these motor units were enhanced or attenuated when performing linear combinations of the signals, as for the representative example in Fig. 5. The ratio (%) between the peak-to-peak amplitude after the linear combination and the peak-to-peak amplitude as recorded at L2 for the 30 motor units was in the range 30.1% to 125.2% (single differential), 7.2% to 122.3% (double differential), and 8.1% to 145.1% (triple differential). Thus some motor unit action potentials were attenuated (ratio < 100%) and others amplified (ratio > 100%) by the linear combination.

The recordings in which signals from the sites L2 and R2 (on the opposite sides of the thin film) were concurrently detected were also analyzed to investigate the effect of a bipolar derivation between the two sides of the thin-film structure. From these recordings, 23 motor units were identified from L2. The ratio (%) between the peak-to-peak amplitude of the action potentials of these motor units as recorded by computing the difference between L2 and R2 with respect to the amplitude when recorded from L2 was in the range 5.2–43.7%, indicating that all motor unit action potentials were attenuated by this differential derivation but to a varying degree, probably depending on the distance from the recording sites.

**DISCUSSION**

This study reports the development and test of a multichannel thin-film wire system for intramuscular EMG recordings.

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**Table 1. Number of identified motor units, peak-to-peak amplitude of the action potentials (range), and noise root mean square value**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Number of MUs (in 4-s intervals)</th>
<th>Peak to Peak (range), μV</th>
<th>Noise RMS† (1 Hz–20 kHz), μV</th>
<th>Noise RMS† (200 Hz–3 kHz), μV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>11 (5*)</td>
<td>40–140</td>
<td>5.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>12 (5*)</td>
<td>53–180</td>
<td>2.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>15 (5*)</td>
<td>20–78</td>
<td>6.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>14 (4*)</td>
<td>36–98</td>
<td>9.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Experiment 5 (first set)</td>
<td>7 (2*)</td>
<td>52–104</td>
<td>3.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Experiment 6 (first set)</td>
<td>8 (2*)</td>
<td>47–68</td>
<td>2.8</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The first 4 experiments consisted of short-term recordings. The last 2 were made of 3 sets of recordings separated by 20 min, of which only the first set is considered in this table (see text for results on the other 2 sets). The noise root mean square (RMS) value is computed without (bandwidth 1 Hz–20 kHz) and with offline digital filtering (bandwidth 200 Hz–5 kHz). MU, motor unit. *Number of epochs of 4-s duration from which the motor unit action potentials were identified. †Computed as average of estimates obtained in 1-s intervals preceding the elicitation of each withdrawal reflex or nerve crush used in the analysis.
The thin-film technology allows the production of multichannel wire systems with small detection sites arranged in a specific geometry. The technology has potential to be applied as a new tool for electrophysiological recordings in muscle.

**Thin-film technology.** Thin-film technology has been recently used as intrafascicular electrodes in peripheral nerves (24). Because the nerve is organized in clusters of topographically related fibers (18), many detection sites allow recording of a subpopulation of fibers (24). There are currently no applications of the thin-fil technology to muscle electrophysiology; however. The present study demonstrates that thin film is a viable technology for intramuscular motor unit recordings. The prototype tested has a thickness of 220 μm, which is larger than that of Teflon-coated stainless steel wires typical for in vivo intramuscular EMG recordings (50–100 μm) but it can be inserted in standard small needles. As a proof of principle, the current prototype consists of eight channels, but the technology implemented allows the extension to a larger number of channels, different interiste distances, and different detection areas. For example, a two-layer thin-film system with 16 electrodes and any interiste spacing would have a width of 280 μm and thickness of 15 μm, that is only slightly larger than the current system. Moreover, this microtechnology allows the combination of the sensors with telemetry and wireless communication (20), which can have applications in myoelectric prostheses controlled by multichannel intramuscular EMG.

Larger number of channels and interiste distance would increase the sampled muscle area and the number of detected sources. For example, a 32-channel system constructed with 2,000-μm intersite spacing would sample from the muscular cross section along 6.2 cm and could be used in large muscles. The same system designed with 400-μm intersite distance would cover 1.24 cm with 32 channels, for use in smaller muscles. A larger site area would reduce the selectivity at the individual recording sites but would decrease the noise level that is directly related to the contact impedance. The flexibility of the design allows the production of systems suited for a variety of applications.

**Multichannel EMG recordings.** Highly selective systems combined with high-density spatial sampling have been adopted for surface EMG detection (25). Multichannel surface EMG is suitable for spatial aspects of motor unit activity, for example to estimate the fiber propagation velocity or the location of the innervation zones (25). A multichannel intramuscular system is, on the other hand, more suited for assessing the temporal characteristics of a group of motor units, i.e., the discharge patterns. Multichannel needle electrodes and multiwire systems have been previously applied (1, 4, 21). In these systems, however, the spatial sampling is used to record the same motor unit activity from slightly different locations to improve the accuracy of action potential classification (1, 8, 21). Alternatively, it is possible to perform subsequent recordings of EMG signals from several needle detection sites (ranging from superficial to deep needle positions) by progressively increasing the insertion depth (16). This technique does not allow concomitant recording of many motor units but does provide separate scans of the muscle electrical activity varying the location of the needle. Previously proposed multichannel, intramuscular detection systems with the aim of spatial sampling (3) were based on needle technology, with the disadvantages of poor stability of the recording, discomfort for the subject, difficulty of application during strong contractions or movement, and poor flexibility in the design of the detection sites.

The thin-film system proposed in this study consists of detection surfaces that have a diameter of 40 μm with distances between detection sites that can be varied from a few hundred micrometers to several millimeters. The small detection sites result in high selectivity that can be further improved by filtering in the time domain (11) or by linear combination of signals detected at the recording sites (Fig. 5). Despite the small detection surface, the experimental tests showed that the signal-to-noise ratio was high enough for discriminating motor unit action potentials (Fig. 4). The signal-to-noise ratio was substantially improved by offline digital filtering of the signal (Table 1), as expected.

The proposed system was also tested for stability. The results on long term recordings showed that the noise level did not substantially change over 40-min intervals and with passive slow movements of the limb. Finally, the system was tested under different sterilization procedures, including autoclaving. No damage to the system was observed after sterilization. The thin-film electrode recorded signals of similar quality with or without autoclaving before the recording (compare experiments 5 and 6 with the first four experiments in Table 1).

**Limitations.** The study reports the construction of a single type of intramuscular thin-film structure. The design of the system is similar to that of thin-film systems for nerve recordings (24). Other design choices may have resulted in a system better optimized for muscle recordings. However, a similar design with respect to nerve recording systems allowed the application of previously tested methods for the connector and insertion procedure (Fig. 2), which was a necessary initial step.

The experimental tests were performed on animal preparations with the skin open and the muscle exposed. The system was inserted into the muscle tissue with a needle that passed through the muscle in two points (Fig. 2). This procedure can be directly applied in human studies with subcutaneous insertion of the thin-film structure (5). The thin-film systems developed for the experiments presented in the present study have been tested under different conditions of mechanical stress after production; thus insertion through the skin would not cause damage to the system. However, the insertion modality used in this study cannot be applied for recordings inside the muscle in vivo, where a different procedure for insertion would have to be implemented. A feasible solution is the construction of a thin-film system whose top part is bent at the tip of the needle, as commonly done with classic wire EMG recordings.

The thin-film system was applied during reflex muscle activity or nerve crushes, during which it is not possible to modulate the number of active motor units, in contrast to the graded activation of the motor unit pool that occurs during voluntary contractions. Ordered activation of individual motor units was not possible with the present experimental procedures. Electrical stimulation of the nerve would have allowed a gradation in the number of activated motor units, but the recorded signals would have had different characteristics with respect to asynchronous motor unit activation. The electrically stimulated signals are deterministic, quasi-periodic signals in which a compound action potential made of the synchronous contributions of a number of motor units repeats with similar shape at each stimulus. The characterization of the system was,
on the contrary, based on the analysis of individual motor unit action potentials repeating in the recording with stochastic characteristics, because it also happens in voluntary contractions.

In summary, this study describes an innovative detection method for single motor unit recordings through a new technology for electrophysiological muscle investigations. The developed electrode can be sterilized for multiple uses and was proven to record signals with stable noise level for up to 40 min.

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GRANTS

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